

- a. combining said specimen with a constant amount of internal reference species (IRS) if the specimen does not already contain one;
- b. capturing and isolating at least one of the one or more certain analytes and said IRS, wherein said capturing and isolating step comprises a substep of combining said IRS containing specimen with an affinity reagent;
- c. quantifying the at least one of the one or more certain analytes in which said quantifying step comprises using mass spectrometric analysis to resolve distinct signals for the analyte and said IRS to determine the amount of the captured analytes relative to the IRS.

49 The method according to claim 48 in which said capturing and isolating step further comprises the steps of:

- a. immobilizing at least one antibody onto a solid substrate to produce an affinity reagent;
- b. combining an effective amount of the affinity reagent with the specimen to produce a post-combination affinity reagent and an unbound remainder;
- c. separating the post-combination affinity reagent from the unbound remainder to form an isolated post-combination affinity reagent;
- d. adding a laser desorption/ionization agent to the isolated post-combination affinity reagent to form a post-combination affinity reagent mass spectrometric mixture.

50 The method according to claim 49 in which said quantifying step further comprises the steps of:

- a. mass spectrometrically analyzing the post combination affinity reagent mass spectrometric mixture to produce a post combination affinity reagent mass spectrum having a mass spectrometric response for the internal reference species located at the unique mass-to-charge ratio of the IRS, and an analyte mass spectrometric response at the unique mass-to-charge ratio of each the certain analyte species thereby detecting the certain analyte species and no mass spectrometric response corresponding to the

mass-to-charge ratio of the certain analyte species when the specimen contains no detectable amount of the analyte species; and

- b. determining whether the amount of the certain analyte species present in the sample is greater or less than the constant amount of the IRS by comparing the mass spectrometric response for detected certain analyte species relative to the mass spectrometric response for the IRS.
- 51 The method of claim 50 wherein the step of combining an effective amount of the affinity reagent with the specimen is accomplished using micropipette tip in which there is a filter element to which the affinity reagent is bound.
- 52 The method of claim 49 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.
- 53 The method of claim 50 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.
- 54 A method for quantifying the relative amount of one or more certain analytes present in a specimen, comprising the steps of:
- a. combining said specimen with a plurality of distinctive internal reference species (IRS's) to the specimen in varied and constant concentrations, each the concentration being chosen to produce a different mass spectrometric response after mass spectrometric immunoassay;
  - b. capturing and isolating at least one of the one or more certain analytes and said plurality of IRS's, wherein said capturing and isolating step comprises a substep of combining said plurality of IRS's containing specimen with an affinity reagent;
  - c. quantifying the at least one of the one or more certain analytes in which said quantifying step comprises using mass spectrometric analysis to resolve distinct

signals for the analyte and said IRS's to determine the amount of the captured analytes relative to the IRS's.

55 The method according to claim 54 in which said capturing and isolating step further comprises the steps of:

- a. immobilizing at least one antibody onto a solid substrate to produce an affinity reagent;
- b. combining an effective amount of the affinity reagent with the specimen to produce a post-combination affinity reagent and an unbound remainder;
- c. separating the post-combination affinity reagent from the unbound remainder to form an isolated post-combination affinity reagent;
- d. adding a laser desorption/ionization agent to the isolated post-combination affinity reagent to form a post-combination affinity reagent mass spectrometric mixture.

56 The method according to claim 55 in which said quantifying step further comprises the steps of:

- a. mass spectrometrically analyzing the post combination affinity reagent mass spectrometric mixture to produce a post combination affinity reagent mass spectrum having a mass spectrometric response for the plurality of IRS's located at the unique mass-to-charge ratio of the IRS's, and an analyte mass spectrometric response at the unique mass-to-charge ratio of each the certain analyte species thereby detecting the certain analyte species and no mass spectrometric response corresponding to the mass-to-charge ratio of the certain analyte species when the specimen contains no detectable amount of the analyte species; and
- b. determining whether the amount of the certain analyte species present in the sample is greater or less than each of the constant amounts of the plurality of IRS's by comparing the mass spectrometric response for detected certain analyte species relative to the mass spectrometric response for the plurality of IRS's.

- 57 The method of claim 55 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.
- 58 The method of claim 56 wherein the step of combining an effective amount of the affinity reagent with the specimen is accomplished using micropipette tip in which there is a filter element to which the affinity reagent is bound.
- 59 The method of claim 56 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.
- 60 The method of claim 59 wherein the step of combining an effective amount of the affinity reagent with the specimen is accomplished using micropipette tip in which there is a filter element to which the affinity reagent is bound.
- 61 The method according to claim 54 in which said quantifying step further comprises interpolating each the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the certain analyte species in the specimen.
- 62 The method according to claim 55 in which said quantifying step further comprises interpolating each the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the certain analyte species in the specimen.
- 63 The method according to claim 56 in which said quantifying step further comprises interpolating each the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the certain analyte species in the specimen.